# Electron Microscopic Changes of Bone Marrow-derived Cultured Mast Cells after Injection into the Skin of Genetically Mast Cell-deficient $W/W^v$ Mice

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Phenotypes of bone marrow-derived cultured mast cells are different from those of connective tissue-type mast cells (CTMCs) that are found in the peritoneal cavity and the skin. When cultured mast cells of $WBB6F_1 - +/+$ mouse origin were directly injected into the skin of genetically mast cell-deficient $WBB6F_1 - W/W^v$ mice, mast cells appeared in both the dermis and the subcutaneous tissue (beneath the *panniculus carnosus*). In contrast to cultured mast cells, mast cells that were observed in either the dermis or the subcutaneous tissue were stained with berberine sulfate, suggesting the content of heparin. Cultured mast cells acquired the electron microscopic features of CTMC in either the dermis or the subcutaneous tissue of $WBB6F_1 - W/W^v$ mice, but the electron density of mast-cell granules was significantly higher in the dermis than in the subcutaneous tissue. Such an electron microscopic difference was also observed after the injection of purified peritoneal mast cells of $WBB6F_1 - +/+$ mice into the skin of $WBB6F_1 - W/W^v$ mice. From the present study, we suggest that the electron density of mast-cell granules in the skin of $WBB6F_1 - W/W^v$ mice is not dependent on the type of injected mast cells but on the anatomical sites at which the injected cells are located. *J Invest Dermatol 91:269–273, 1988*

Cells with many features of mast cells develop in cultures of mouse hematopoietic cells containing interleukin 3 [1–11]. Large numbers of cells ($> 10^8$) can be generated as virtually homogeneous populations or as clones, and thus a wide variety of biochemical and functional studies of cultured mast cells are facilitated [12].

Cultured cells differ in certain important respects from the best-characterized cell type available for detailed comparative studies: mature peritoneal mast cells (reviewed in Ref 12). Cultured mast cells appear immature by ultrastructure [1,2,12], contain low levels of histamine [1,2,5,12–15], and express fewer surface receptors for IgE than do mature peritoneal mast cells. In addition, cultured mast cells incorporate $Na_2\,^{35}SO_4$ into granule-associated chondroitin sulfate [2,10,14,16], including an oversulfated chondroitin sulfate E [10,16]. By contrast, normal mouse peritoneal mast cells synthesize heparin [2,16].

However, peritoneal mast cells are representative of only one of two phenotypically distinct mast cell populations that have been identified in mice and rats. Maximow [17] was probably the first to recognize that staining characteristics of certain mast cells in the rat intestinal mucosa differed from those of mast cells observed in other anatomical sites. Enerbäck [18] greatly extended the observation and defined conditions of fixation and histochemical staining that discriminated between such mucosal mast cells (MMCs) and the connective tissue-type mast cells (CTMCs) of the peritoneal cavity, the skin, and other sites.

Cultured murine mast cells express certain characteristics similar to those of MMCs. MMCs contain low levels of histamine [18,19], and their granules contain chondroitin sulfate [20,21]. Cultured mast cells and MMCs also exhibit similar patterns of responsiveness to certain secretagogues [14] and, in the rat, contain similar or identical granule-associated protease [22].

Although some authors have suggested that cultured mast cells may be committed to express the MMC phenotype, we have recently shown that cultured mast cells may acquire the CTMC phenotype when they are transferred into the peritoneal cavity of $W/W^v$ mice that are genetically deficient in mast cells [23]. Cultured mast cells do not stain with berberine sulfate, but mast cells recovered from the peritoneal cavity of $W/W^v$ mice stain with this fluorescent dye that binds heparin. This suggests that the adoptively transferred mast cell population acquired the ability to synthesize and store heparin. In fact, Otsu et al [24] recently demonstrated with biochemical techniques that mast cells recovered from the peritoneal cavity of $W/W^v$ mice synthesized heparin proteoglycan. After intraperitoneal transfer, the content of histamine increased by a factor of 20 [23], and Forssman antigens appeared on the surface of mast cells [24].

In the present study, we investigated the process of the phenotypic change with the electron microscope. When cultured mast cells were subcutaneously injected into the skin of $W/W^v$ mice, CTMCs appeared in two different sites of the skin; the dermis and

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Abbreviations:

CTMC: connective tissue-type mast cell

MMC: mucosal mast cell

PWM-SCM: pokeweed mitogen-stimulated spleen cell-conditioned medium

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the subcutaneous tissue (i.e., beneath the *panniculus carnosus*). Unexpectedly, the electron microscopic features of mast cells that appeared in these two anatomical sites of the skin tissue were different from each other. We will hereafter describe the electron microscopic changes of cultured mast cells, which are probably influenced by anatomical location of the injected cells.

## MATERIALS AND METHODS

**Mice** The tissues of adult WBB6F$_1$ — $W/W^v$ mice contained <1% the number of CTMCs as do their normal (+/+) littermates [25,26]; WBB6F$_1$ — $W/W^v$ mice also lack MMCs [27]. WBB6F$_1$ — $W/W^v$ and —+/+ mice were raised at the Shizuoka Laboratory Animal Center. Cells derived from C57BL/6 — $bg^J/bg^J$ mice were used in an experiment because their giant granules represent a morphologic marker that can unequivocally identify the cells as of donor origin [28,29]. The original stocks of mutant mice were obtained from the Jackson Laboratory (Bar Harbor, ME), but the $W^v$ and $bg^J$ mutant genes have been maintained in C57BL/6 mice of our own inbred colony [30,31]. Mice were used at 2–6 months of age.

**Cultured Mast Cells** Bone marrow cells of either WBB6F$_1$ — +/+ or C57BL/6 — $bg^J/bg^J$ mice were recovered and suspended in Eagle's medium as described previously [32], and were cultured in the presence of pokeweed mitogen-stimulated spleen cell-conditioned medium (PWM-SCM) [23,33].

**Peritoneal Mast Cells** Separation of peritoneal mast cells was carried out by using the method described by Yurt et al [34] with a slight modification [35]. Mast cell suspensions of >99% purity were used for injection.

**Cell Injection** Cultured or peritoneal mast cells were counted in a standard hemocytometer and were injected into the skin of WBB6F$_1$ — $W/W^v$ mice. To facilitate direct injections of cells into the skin, an area of dorsal skin was shaved with hair clippers. Cells ($10^4$ cells in 0.05 ml Eagle's medium per site) were injected with a tuberculin syringe. Each mouse received six injections, which were marked by mixing India ink with the suspension medium. WBB6F$_1$ — $W/W^v$ mice were killed at various days after the direct injections of either cultured or peritoneal mast cells. The dorsal skin was reflected; each injection site that could be identified by the presence of India ink was removed; and skin pieces were embedded in paraffin. Specimens stained with berberine sulfate were examined with an Olympus epifluorescence microscope [23,36]. In some cases, the specimens were stained with alcian blue after the observation with the epifluorescence microscope [23]. The experiment was repeated three times.

**Electron Microscopy** Specimens were fixed for 60 min at 4°C in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4, and then postfixed for 90 min at 4°C in 1% osmium tetroxide in 0.1 M phosphate buffer at pH 7.4. Specimens were dehydrated with ethanol and embedded in Epon 812. Ultrathin sections were obtained with an LKB ultramicrotome, and were stained with uranyl acetate and lead citrate. The sections were observed in a JEM-100 electron microscope at an accelerating voltage of 80 kv.

## RESULTS

Bone marrow-derived cultured mast cells ($10^4$) of WBB6F$_1$ — +/+ mouse origin were injected into the skin of genetically mast cell-deficient WBB6F$_1$ — $W/W^v$ mice; the recipient mice were killed 4, 8, and 16 weeks after the injection. Clusters of mast cells appeared in either the dermis or the subcutaneous tissue (beneath the *panniculus carnosus*) (Fig 1). Mast cells that appeared in either site were stained with berberine sulfate, suggesting the content of heparin. Such mast-cell clusters were not observed in the skin of nontreated WBB6F$_1$ — $W/W^v$ mice. Moreover, when cultured mast cells of C57BL/6 — $bg^J/bg^J$ mouse origin had been injected, all mast cells in the clusters had giant granules. Therefore, we considered that the appearance of mast cell clusters resulted from the injection of cultured mast cells.

Although electron microscopic features of cultured mast cells

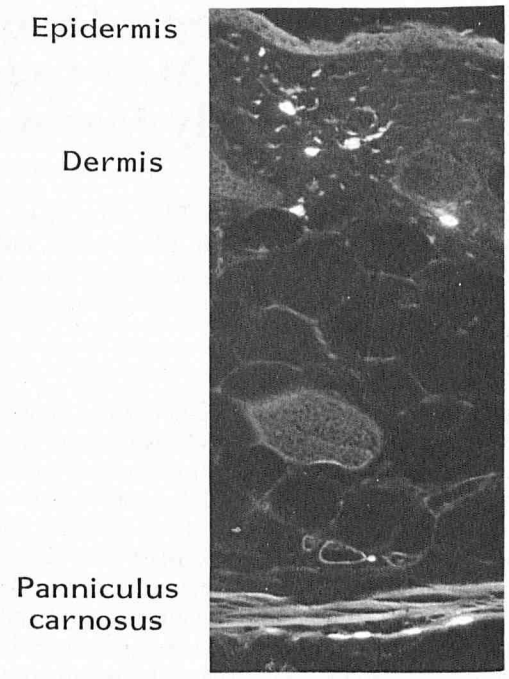


**Figure 1.** Mast cells that were observed in the skin of WBB6F$_1$ — $W/W^v$ mice 16 weeks after the injection of cultured mast cells. Berberine sulfate-positive mast cells appeared in either the dermis or the subcutaneous tissue (beneath the *panniculus carnosus*). Stained with berberine sulfate (×720).

(Fig 2*A*) differed from those of CTMCs as described by Galli et al [2,12] and Dvorak et al [37], cultured mast cells gradually acquired the electron microscopic features of CTMCs after the injection into the skin of WBB6F$_1$ — $W/W^v$ mice. Granules with dense progranular material disappeared, and most granules were homogeneously electron dense 8 weeks after the injection. Although the change was observed in either the subcutaneous tissue or the dermis, the electron density of mast cell granules was significantly lower in the former tissue (Figs 2*B*, *D*) than in the latter tissue (Fig 2*C*). All mast cells examined 12 weeks after the injection were very similar in either the dermis or the subcutaneous tissue. In other words, granules of all examined mast cells were homogeneously dense in the dermis, and were homogeneously less dense in the subcutaneous tissue.

In the next experiment, peritoneal mast cells (Fig 3*A*) of WBB6F$_1$ — +/+ mice were purified and injected into the skin of WBB6F$_1$ — $W/W^v$ mice ($10^4$ cells per each injection site). As described after the injection of cultured mast cells, mast cells appeared in either the subcutaneous tissue or the dermis. The electron density of mast cell granules was lower in the former tissue (Figs 3*B*, *D*) than in the latter tissue (Fig 3*C*). Granules of all mast cells examined 12 weeks after the injection were homogeneously dense in the dermis, and were homogeneously less dense in the subcutaneous tissue.

As a result, the dermal mast cells that were observed at the injection sites of cultured mast cells (Fig 2*C*) appeared similar to those that were observed at the injection sites of peritoneal mast cells (Fig 3*C*). The subcutaneous mast cells that were observed at the injection sites of cultured mast cells (Fig 2*D*) were also similar to those observed at the injection sites of peritoneal mast cells (Fig 3*D*).

Electron microscopic features of mast cells in the skin of intact WBB6F$_1$ — +/+ mice were investigated to compare with those of cultured or peritoneal mast cells transplanted into the skin of WBB6F$_1$ — $W/W^v$ mice. Electron microscopic features of mast cells in the dermis of intact WBB6F$_1$ — +/+ mice were indistinguishable from those of mast cells that were observed in the dermis of WBB6F$_1$ — $W/W^v$ mice 8 weeks after the injection of either cultured or peritoneal mast cells. We also attempted to investigate

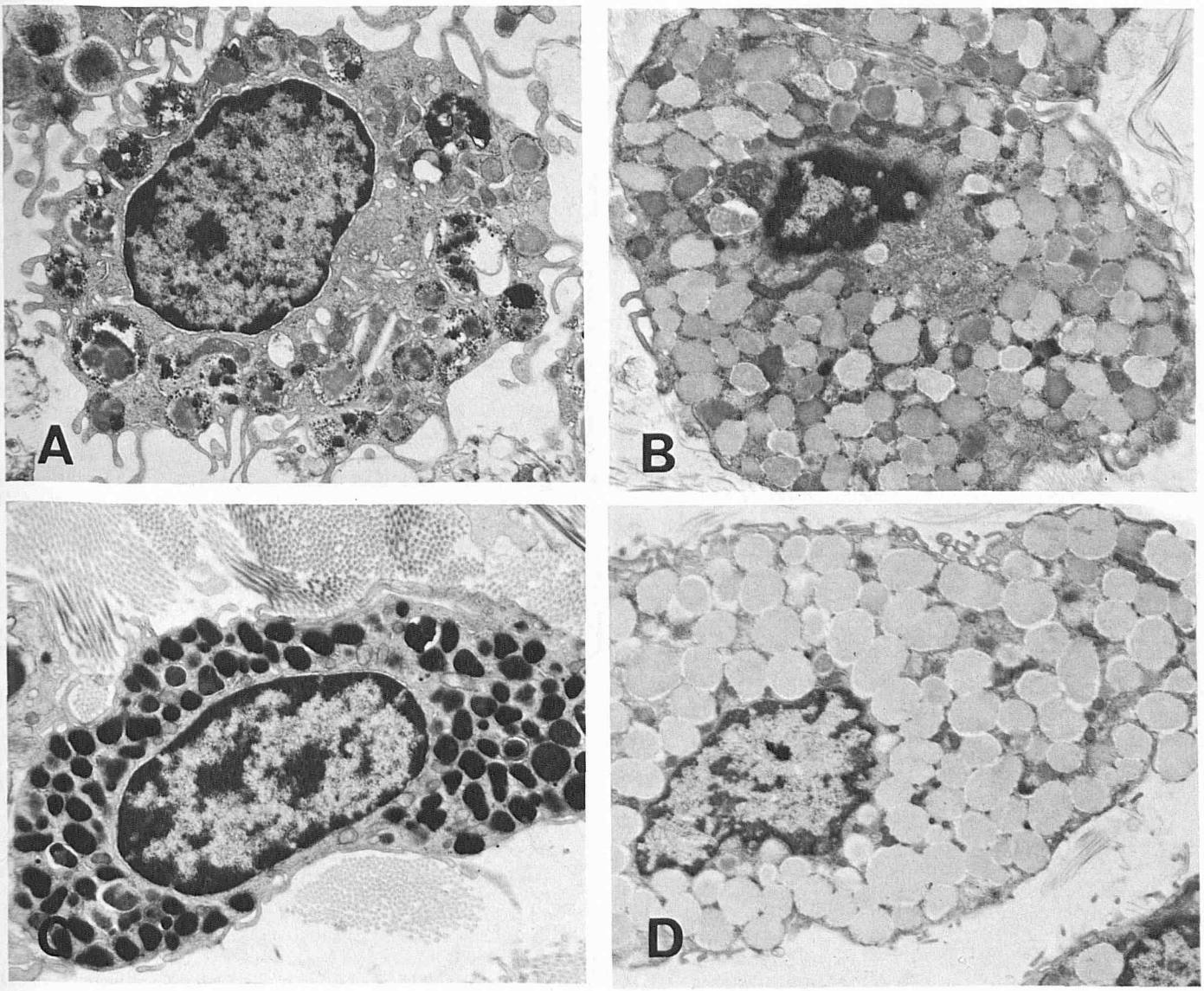


**Figure 2.** Electron microscopic changes of cultured mast cells after the injection into the skin of WBB6F$_1$ − *W/W$^v$* mice. *A*: a cultured mast cell. *B* and *D*: mast cells that were observed in the subcutaneous tissue of WBB6F$_1$ − *W/W$^v$* mice at 8 (*B*) and 16 (*D*) weeks after the injection of cultured mast cell. *C*: a mast cell that was observed in the dermis of a WBB6F$_1$ − *W/W$^v$* mouse 8 weeks after the injection of cultured mast cells, of which electron microscopic features did not change thereafter (×7200).

mast cells in the subcutaneous tissue of intact WBB6F$_1$ − +/+ mice. However, the concentration of mast cells was much less in the subcutaneous tissue than in the dermis. We prepared 36 blocks for electron microscopy from 12 WBB6F$_1$ − +/+ mice and extensively searched subcutaneous mast cells, but we could find none of them in these 36 blocks.

## DISCUSSION

After the injection of bone marrow-derived cultured mast cells into the skin of genetically mast cell-deficient WBB6F$_1$ − *W/W$^v$* mice, clusters of mast cells were observed in either the dermis or the subcutaneous tissue. Mast cells of both anatomical sites stained with berberine sulfate. Enerbäck demonstrated that berberine sulfate specifically stained heparin-containing granules of CTMCs by cytofluorometry [35], and we recently confirmed this by showing that CTMC granules did not stain with berberine sulfate after exposure of the cells to heparinase digestion [23]. Moreover, biochemical analysis of mast cell proteoglycans indicates that berberine sulfate-positive mast cells incorporate $Na_2{}^{35}SO_4$ predominantly into heparin, whereas berberine sulfate-negative cultured mast cells incorporate $Na_2{}^{35}SO_4$ predominantly into chodroitin sulfate [24]. Therefore, berberine sulfate-positive mast cells that appeared in either the dermis or the subcutaneous tissue were considered to be heparin-containing CTMCs.

However, electron microscopic features of mast cells in the dermis were different from those of the subcutaneous tissue; the electron density of granules was higher in the former than in the latter. In contrast to the bone marrow-derived cultured mast cells, peritoneal mast cells are typical CTMCs. The electron density of granules in mast cells that appeared in the dermis after the injection of peritoneal mast cells was comparable to that of the original peritoneal mast cells. However, the electron density of granules in mast cells that were observed in the subcutaneous tissue after the injection of peritoneal mast cells was lower than that of granules of the original peritoneal mast cells, and was comparable to that of granules of mast cells that were observed in the subcutaneous tissue after the injection of cultured mast cells. Therefore, the electron density of granules was not dependent on the type of injected mast cells but the anatomical sites in which mast cells were present.

Although mast cells that developed in either the dermis or the subcutaneous tissue stained with berberine sulfate, electron micro-

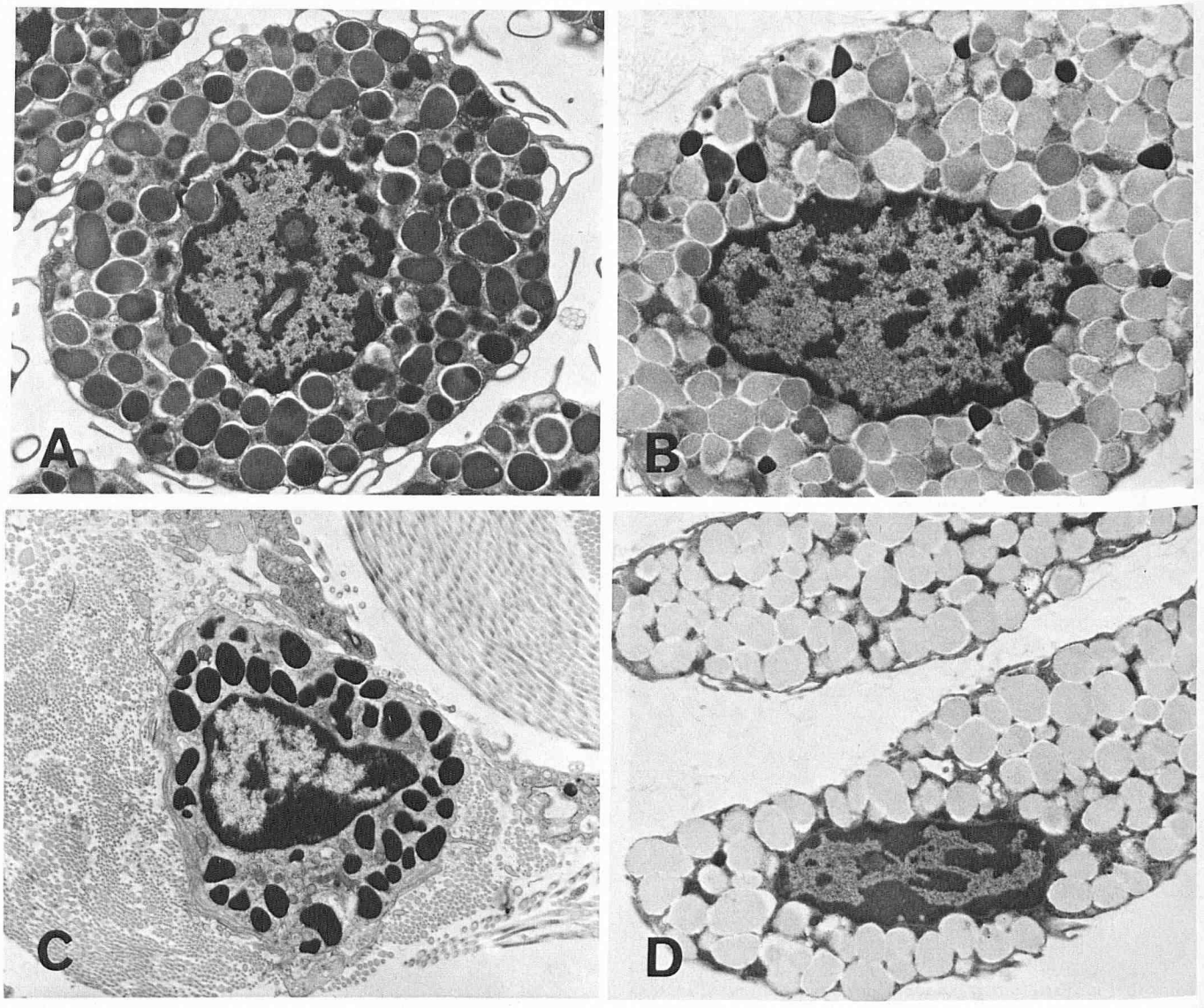


**Figure 3.** Electron microscopic changes of peritoneal mast cells after the injection into the skin of WBB6F$_1$ — $W/W^v$ mice. *A*: a peritoneal mast cell. *B* and *D*: mast cells that were observed in the subcutaneous tissue of WBB6F$_1$ — $W/W^v$ mice at 8 (*B*) and 16 (*D*) weeks after the injection of peritoneal mast cells. *C*: a mast cell that was observed in the dermis of a WBB6F$_1$ — $W/W^v$ mouse 8 weeks after the injection of peritoneal mast cells, of which electron microscopic features did not change thereafter (×7200).

scopic features of the dermal mast cells were different from those of subcutaneous mast cells. Thus, the electron microscopy appears to be the more sensitive method for characterization of CTMCs than the staining with berberine sulfate.

Morphologic differences among CTMCs have been described by Aldenborg and Enerbäck [38]. They histochemically demonstrated that the subepithelial mast cells of the rat skin contained heparin proteoglycan, which is, however, different from that of typical CTMCs observed in the deep dermis. Moreover, the electron microscopic examination showed that the number of granules was less in subepithelial mast cells than in mast cells in the deep dermis [38]. We also attempted to detect the electron microscopic difference between dermal and subcutaneous mast cells in the skin of intact WBB6F$_1$ — +/+ mice. However, we could not obtain electron micrographs of mast cells in the subcutaneous tissue of WBB6F$_1$ — +/+ mice due to their low concentration. There is a possibility that the subcutaneous tissue of the mouse is not appropriate for development and/or maintenance of CTCMs. If this is the case, CTCMs with less dense granules, which were observed in the subcutaneous tissue of WBB6F$_1$ — $W/W^v$ mice after the injection of either cultured or peritoneal mast cells, may represent a slowly dying population in this inappropriate environment.

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